Standard Operating Procedure For the Determination of Polycyclic Aromatic Hydrocarbons (PAHs) by Liquid-Liquid Extraction and High Performance Liquid Chromatography

1.0 Purpose

1.1 The purpose of this standard operating procedure (SOP) is to allow for the qualitative and quantitative determination of polycyclic aromatic hydrocarbons (PAH) that may be present in water.

2.0 Scope

2.1 The PAH analysis is achieved by combining liquid-liquid extraction and High Performance Liquid Chromatography (HPLC). This method is modeled after the Environmental Protection Agency (EPA) Method 550.[1] PAHs are extracted from the water matrix with methylene chloride by liquid-liquid extraction. The PAHs are then concentrated and acetonitrile is added to the extract. The extract is then concentrated and brought to volume with reagent water. It is then injected on the HPLC system and detected by ultraviolet (UV) detection. This SOP is applicable to water samples such as drinking water, ground water, and surface water. The compounds that may be analyzed by this procedure are:

Analyte	Analyte Number	
Acenaphthene	30695	
Acenaphthylene	30705	
Anthracene	30710	
Benzo(a)anthracene	30720	
Benzo(a)pyrene	30690	
Benzo(b)fluoranthene		30725
Benzo(g,h,i)perylene	30730	
Benzo(k)fluoranthene		30735
Chrysene	30740	
Dibenzo(a,h)anthracene	30755	
Fluoranthene	30760	
Fluorene	30765	
Indeno(1,2,3-cd)pyrene	30770	
Naphthalene	30700	
Phenanthrene	30775	
Pyrene		30780

The EPA only regulates benzo(a)pyrene; therefore, this SOP will only address benzo(a)pyrene. The method detection limit for benzo(a)pyrene is 0.02 ug/L.

Benzo(a)pyrene (analyte number 30690)

3.0 Safety

3.1 The toxicity and carcinogenicity of each reagent used in this method have not been precisely defined; however, each chemical compound should be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. Material safety data (MSD) sheets should be on file for all analytes and reagents.[1]

3.2 The following analytes have been tentatively classified as known or suspected, human or mammalian carcinogens: benzo(a)anthracene, benzo(a)pyrene, and dibenzo(a,h)anthracene. Primary standards of these toxic compounds should be prepared in a hood.[1]

4.0 Interferences

4.1 Method interferences may be caused by contaminants in the solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts and/or elevated baselines in the chromatograms. The method must be demonstrated to be free from interferences by running solvent blanks and laboratory blanks with each analysis. Several potential sources of interferences are listed below.[1]

4.1.1 Glassware must be thoroughly cleaned. Wash the glassware with water and detergent followed by rinsing with distilled water. Allow the glassware to dry and rinse with acetone and hexane.[1]

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems.

4.1.3 Interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source.[1]

5.0 Sample Collection, Preservation, and Handling

5.1 Grab samples must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle must not be pre-rinsed with the sample before collection.[1]

5.2 All samples must be iced or refrigerated at 4°C from the time of collection until extraction. PAHs are known to be light sensitive; therefore, samples,extracts, and standards should be protected from the light.

5.3 Samples which are known or suspected to contain residual chlorine must be preserved with sodium thiosulfate (100 mg/L).[1]

5.4 Samples must be extracted within 7 days of collection. Extracts must be analyzed within 30 days of extraction.[1]

6.0 Chemicals and Stock Solutions

6.1 Chemicals

6.1.1 Reagent water: For use in the HPLC mobile phase and in the preparation of other reagents. Reagent water is defined as water that is reasonably free of contamination that would prevent the determination of any analyte of interest. Distilled water purified by a Barnstead Nanopure II system is suitable for this procedure. The water from the Barnstead system must be filtered through 0.45 uM filters using mechanical means to remove microparticulates that are detrimental to the HPLC system.

6.1.2 Sodium thiosulfate: ACS grade or better

6.1.3 Methylene Chloride: Pesticide quality or equivalent

6.1.4 Acetonitrile (CH_3CN): HPLC quality

6.2 Stock Solution

6.2.1 Stock standards can be prepared from dry, pure powder or can be purchased from several vendors at certified concentrations.

6.2.1.1 Preparation from dry, pure powder: According to EPA Method 550, accurately weigh and dissolve 0.0100 g of pure material (96%or greater purity) in 10 ml of acetonitrile. The concentration of this stock solution will be 1.00 ug/ul. Primary standards should be prepared in the hood.[1]

6.2.1.2 Preparation from commercially prepared solutions: PAH mix (all 16 analytes) is available from several commercial sources. Prepare dilutions as necessary for the analysis. See section 9.2 and 9.4 for the details on standard preparation.

6.2.2 Internal standard, p-Terphenyl, can be prepared from dry powder or can be purchased through several vendors at certified solutions. It is recommended to use the certified solutions. See section 9.3 and 9.4 for details on the preparation.

7.0 Equipment and Apparatus

7.1 Analytical equipment

7.1.1 Varian 9010 solvent delivery system with Membrane Degasser or an equivalent system that is capable of delivering a gradient mobile phase at a constant flow

7.1.2 TSP SpectraSYSTEM AS3000 autosampler or an equivalent automatic sampling system capable of delivering consistent injection volumes ranging from 100 ul to 1000 ul

7.1.3 LDC Analytical SM5000 or an equivalent detection system capable of monitoring ultraviolet (UV) absorption at various wavelengths.

7.1.4 Fiatron CH-30 oven and TC-50 temperature controller or an equivalent system capable of maintaining a constant elevated temperature for the HPLC column

7.2 Extraction Apparatus

7.2.1 Separatory funnels: 2L, with Teflon stopcocks

7.2.2 Zymark flask: 250 ml

7.2.3 Turbo Vap II Concentration Workstation

8.0 Sample Extraction Procedure

8.1 Pour 1 L of the sample into a separatory funnel.

8.2 All samples must contain the internal spike, p-Terphenyl. The preparation is discussed in section 9.3.

8.2 The lab must analyze one lab fortified blank (blank spike) and one fortified sample (sample spike) with every 10 samples or one per sample set, whichever is greater. Using the benzo(a)pyrene and p-Terphenyl solution (see section 9.5), make the appropriate dilution into the separatory funnel.

8.3 Add 60 ml of methylene chloride to each of the separatory funnels. Extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase. Collect the methylene chloride extract in a 250 ml Zymark flask.[1]

8.4 Add a second 60 ml volume of methylene chloride to the separatory funnel and repeat the extraction procedure, combining the extracts in the Zymark flask. Perform a third extraction in the same manner.[1]

8.5 Place the Zymark flask in the Turbo Vap II Concentration Workstation. Evaporate methylene chloride extract to 1 ml. Add 10 ml of acetonitrile and concentrate to a volume of 1 ml. Add another 10 ml of acetonitrile and concentrate to a volume of 1 ml. Dilute to 4 ml volume with filtered reagent water.

9.0 Analytical Procedure

9.1 Analytical condition

9.1.1 HPLC column: Vydac 201TP (5 u, C18, 4.6 X 250 mm)[2]
Part # 201TP54 or any other column proven to work.

9.1.2 HPLC guard column: in-line filter

9.1.3 HPLC mobile phase: gradient

A: reagent water B: acetonitrile C: acetonitrile

Note: Two reservoirs must be filled with acetonitrile due to the amount needed for the gradient mobile phase.

Time	% A	% B	% C
0	65	18	17
2	65	18	17
24	0	50	50
30	0	50	50
35	65	18	17
45	65	18	17

9.1.4 Flow: 2.0 ml/min

9.1.5 Method run time: 45 minutes

9.1.6 Data collection time: 30 minutes

9.1.7 Autosampler parameters:

Injection volume: 500 ul
Run time: 43 minutes

All other parameters can remain at default values.

9.1.8 Column temperature: 30°C

9.1.9 Ultraviolet detector parameters: absorption at 254 nm, 295 nm

Note: The recommended wavelength for benzo(a)pyrene is 254 nm; however, the wavelength 295 nm is preferred. The absorption of the interference peak in the benzo(a)pyrene window is decreased at 295 nm.

9.2 Standard stock preparation

9.2.1 The commercially available PAH mix (all 16 analytes) is typically at a concentration of 2000 ug/ml. (TCL PAH mix # 4-8905M from Supelco)

9.2.2 Prepare a stock solution of all 16 analytes in acetonitrile from the commercial standard as follows:

PAH Stock: 2000 ug/ml X 50 ul/10000 ul = 10 ug/ml

9.3 Internal standard stock preparation

9.3.1 The commercially available p-Terphenyl is typically at a concentration of 1000 ug/ml. (Absolute Standard # 71227)

9.3.2 Prepare a internal standard stock solution of p-Terphenyl in acetonitrile from the commercial standard as follows:

p-Terphenyl Stock: 1000 ug/ml X 50 ul/5000 ul = 10 ug/ml

9.3.3 All samples must contain the internal standard, p-Terphenyl. The samples are prepared by adding 20 ul of the internal standard to the sample in the

separatory funnels. The internal standard concentration is 0.2 ug/L.

9.4 Analytical standard preparation

9.4.1 The analytical standards are prepared with the PAH stock solution (all 16 analytes) in H_2O/CH_3CN (70/30) as follows:

Standard 1: 10 ug/ml X 5 ul/10000 ul = 0.005 ug/ml
Standard 2: 10 ug/ml X 5 ul/5000 ul = 0.01 ug/ml
Standard 3: 10 ug/ml X 25 ul/5000 ul = 0.05 ug/ml
Standard 4: 10 ug/ml X 50 ul/5000 ul = 0.1 ug/ml

All standards should be prepared with the internal standard p-Terphenyl at a concentration of 0.005 μg/ml. (25μl/5000μl)

9.4.2 The method detection limit (MDL) for benzo(a)pyrene is 0.02 ug/L. The dilution factor for all samples is 4 because all the samples have a final volume of 4 ml. The MDL is achieved as follows:

Standard 1 = 0.005 ug/ml X 4 ml = 0.02 ug/L

9.5 Spike preparation

9.5.1 The commercially available benzo(a)pyrene is typically at a concentration of 1000 ug/ml. A stock spike solution is prepared in acetonitrile as follows:

Spike stock: 1000 ug/ml X 100 ul/10000 ul = 10 ug/ml

9.5.2 The lab must analyze one lab fortified blank (blank spike) and one fortified sample (sample spike) with every 10 samples or one per sample set, whichever is greater. The lab fortified blank and lab fortified sample are prepared with the spike stock solution and the p-Terphenyl stock solution by adding 20 ul to the spike sample in the separatory funnels. The spike concentration is 0.2 ug/L.

9.6 Sample preparation

The samples must be extracted within 7 days of collection. The extracts must be analyzed within 30 days of the extraction.

9.7 HPLC setup and equilibration

Turn on the HPLC system components. Flush the column with a 50/50 mixture of

reagent water/acetonitrile. Then flush the column with a 10/90 mixture of reagent water/acetonitrile. Follow this with a brief 50/50 flush to ease the transition to the next step. Lastly, flush the column with a 90/10 mixture of reagent water/acetonitrile. When the flushing has been completed, begin pumping the initial conditions for the mobile phase through the column.

9.8 HPLC analysis

Transfer the standards and the samples to autosampler vials. The standards should be injected at the beginning and end of the analysis. If a large number of samples are to be analyzed, standards may be injected in the middle of the analysis. Computer setup and use of software will not be included in this SOP. It will be left to the analyst to determine the procedures as the procedures vary from instrument to instrument.

9.9 HPLC shutdown

When the analysis has been completed, several steps are necessary to prepare the system for shutdown. Flush the column with a 90/10 mixture of reagent water/acetonitrile until about 30 to 40 ml of solvent has been flushed through the column. Briefly, flush the column with a 50/50 mixture of reagent water/acetonitrile. Then flush with a 10/90 mixture of reagent water/acetonitrile until about 30 to 40 ml of solvent has been flushed through the column. Finally, flush the column with a 50/50 mixture of reagent water/acetonitrile. The column is stored in the 50/50 mixture.

10.0 Data Analysis

10.1 Identification of p-Terphenyl and benzo(a)pyrene

10.1.1 Identification of p-Terphenyl and benzo(a)pyrene is done by comparing the retention times of the sample to the standard injections. The retention time of p-Terphenyl is typically around 20 minutes. The retention time of benzo(a)pyrene is typically around 25 minutes. HPLC analytes are subject to retention time shifts. The window of acceptability is usually ±20 seconds of the average standard retention time.

10.1.2 Typical chromatograms are included at the end of this SOP.

10.2 Calculations of results

The computer software is typically used to calculate results. The procedures will vary depending upon the system used. It will be left to the analyst to implement the

software.

11.0 Quality Control

11.1 Quality control consists of the analysis of laboratory reagent blanks, solvent blanks, laboratory fortified blanks, and laboratory fortified sample.[1]

11.1.1 Laboratory reagent blanks and solvent blanks: A laboratory reagent blank (lab blank) and solvent blank must be analyzed with each set of samples. If within the retention time window of benzo(a)pyrene the lab blank and/or solvent blank produces a peak that would prevent the determination of benzo(a)pyrene, determine the source of the contamination and eliminate the interference before processing the samples.[1]

11.1.2 Laboratory fortified blanks: The analyst must analyze at least one laboratory fortified blank (blank spike) with every 10 samples or one per sample set, whichever is greater.[1] The spiking level should be 10 times the method detection limit (MDL), which is currently 0.02 ug/L. The spike should be prepared along with the sample extractions. See section 9.5 for the basic spike preparation procedure.

11.1.3 Laboratory fortified samples: The analyst must analyze at least one laboratory fortified sample (sample spike) with every 10 samples or one per sample set, whichever is greater.[1] The spiking level should be 10 times the method detection limit (MDL), which is currently 0.02 ug/L. The spike should be prepared along with the sample extractions. See section 9.5 for the basic spike preparation procedure.

11.1.4 Control limits and acceptability of results for laboratory fortified blanks (blank spike) and laboratory fortified samples (sample spike) are as follows:

11.1.4.1 Control limits: Control limits are determined by calculating the mean percent recovery (X) and the standard deviation (S) of the percent recovery. The equations for the upper and lower limits are[1]:

Upper limit = X + 3S
Lower limit = X - 3S

11.1.4.2 Acceptability of results: Any benzo(a)pyrene spike result whose percent recovery for that set of samples is outside the control limits cannot be reported. The set must be reanalyzed with a new spike or not reported.

11.2 System suitability: No formal system suitability is done on a daily basis. The standards are evaluated as they become available. Generally, if the benzo(a)pyrene retention time is similar to that of the previous analysis, peak shape is good, and the minor peak that may appear in the benzo(a)pyrene window is not interfering with the benzo(a)pyrene, the system is considered suitable for the analysis. HPLC system pressures are recorded at the beginning of each analysis and compared to previous readings to detect changes that indicate system problems.

12.0 Documentation

12.1 Results are typically recorded in a notebook.

12.2 Compare the quality control samples to their control limits. Record the spike results and note if they pass or fail to indicate result acceptability.

12.3 Computer files: The computer files should be transferred to diskettes and stored for future reference. After the files have been transferred, they can be deleted from the computer hard drive.

12.4 Hard copies of the reports must be filed.

13.0 References

13.1 EPA 550 Determination of Polycyclic Aromatic Hydrocarbons in Drinking Water by Liquid-Liquid Extraction and HPLC with Coupled Ultraviolet and Fluorescence Detection. (1990)

13.2 “New Columns for PAH Analysis,” Hewlett Packard Peak, Number 4 1992